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Laboratory Experiment

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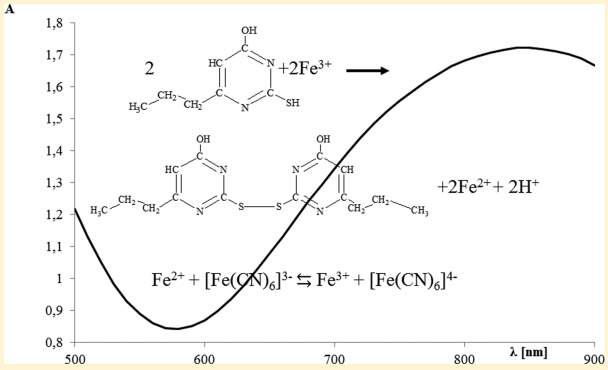
Spectrophotometric Determination of 6-Propyl-2-thiouracil in Pharmaceutical Formulations Based on Prussian Blue Complex Formation: An Undergraduate Instrumental Analysis Laboratory Experiment

Robert Zakrzewski,* Monika Skowron, Witold Ciesielski, and Żaneta Rembisz

Faculty of Chemistry, University of Łódź, Tamka 12, 91-403 Łódź, Poland

Supporting Information

ABSTRACT: The laboratory experiment challenges students to determine 6-propyl-2-thiouracil (PTU) based on Prussian blue complex formation. Prussian blue is formed by ferricyanide and Fe(II) ions which are generated in situ from Fe(III) ions reduced by PTU. The absorbance of this product was measured at a wavelength of 840 nm, after a reaction time of 30 min. The range of determination was 0.34−3.4 μg mL^{-1} of PTU per sample. The method was successfully used in an upper-division instrumental analysis laboratory course to determine the concentration of PTU in commercially available pharmaceuticals. Additionally, students in introductory or high school chemistry classes by conducting this experiment will gain a greater understanding of the chemical analysis process, redox reactions, and the Beer−Lambert law.



KEYWORDS: *Upper-Division Undergraduate, Analytical Chemistry, Hands-On Learning/Manipulatives, Drugs/Pharmaceuticals, Instrumental Methods, Quantitative Analysis, UV-Vis Spectroscopy*

INTRODUCTION

6-Propyl-2-thiouracil (PTU) shown in Figure 1 is applied as an active compound in drugs used for the treatment of human hyperthyroidism caused by thyroid gland hyperfunction.[1,2]

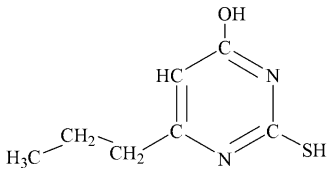
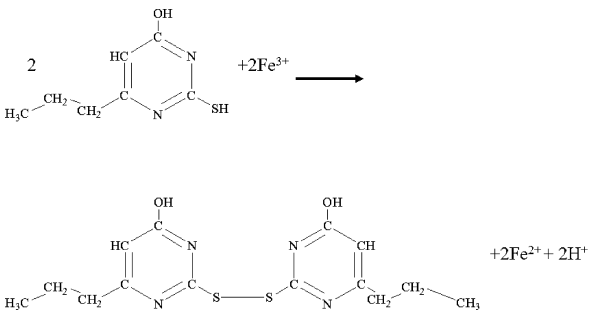


Figure 1. Structure of 6-propyl-2-thiouracil.

PTU can reduce Fe(III) ions (eq 1):



(1)

The next step is oxidation of iron(II):

$$Fe^{2+} + [Fe(CN)_6]^{3-} \leftrightarrows Fe^{3+} + [Fe(CN)_6]^{4-} \quad (2)$$

and Prussian blue ($Fe_4[Fe(CN)_6]_3$) is formed (eq 3, ref 3):

$$4Fe^{3+} + 3[Fe(CN)_6]^{3-} \rightarrow Fe_4[Fe(CN)_6]_3 \quad (3)$$

The amount of the resulting Prussian blue is proportional to the amount of PTU. Thus, the amount of PTU can be calculated based on the absorbance of the blue complex measured at the maximum absorption wavelength.

Several spectrophotometric methods have been previously reported for determining the presented compound in pharmaceutical forms. Color reactions of 6-propyl-2-thiouracil with palladium(II) chloride (λ = 525 nm)[4] and potassium iodate (λ = 465 nm)[5] have been utilized for PTU determination as well. A kinetic method based on the inhibitory effect of PTU on the Pd(II)-catalyzed reaction of neutral red[6] and crystal violet[7] with hypophosphite ions has also been developed.

This paper presents the application of the Prussian blue complex reaction in spectrophotometric determination of 6-propyl-2-thiouracil in pharmaceutical preparations.

The paper describes the development of the experiment and presents the data obtained by the authors. Students gain practical experience in using a centrifuge, an analytical balance, and a spectrophotometer, as well as in applying the Beer−Lambert−Bouguer law. In the advanced experiment, students

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J. Chem. Educ. XXXX, XXX, XXX−XXX

also practice creating graphs, determining the slope of a line, and using calibration curves. The lab time required is 2 or 3 h. Also, the procedure is appropriate for high school or general science classes.[8]

Greater emphasis on acquiring new skills in the process of learning is consistent with the modern tendencies. Various fields of education now tend to accentuate the need to develop and improve complex skills, such as argumentation, reasoning, or drawing conclusions. So far, the focus was very often on isolated skills, as a result of which teachers were encouraged to teach and practice these practical skills separately. However, that was not conducive to instilling creative and critical thinking or problem solving abilities. Teachers should remember that complex skills are not complex just because they comprise many components, but because of the complex relationships between those components. The development of complex skills is a long-term process and requires considerable competence, and especially knowledge about critical thinking.[9]

Of great importance for the development and training of complex skills is the practice of performing chemical determinations by students. Such determinations not only involve many analytical manual steps (e.g., weighing, dilution, preparation of solutions, measurement of physicochemical parameters), but students must consider how particular elements are related: analyze and compare them, and identify cause-and-effect relationships. Another competence is the application of the knowledge gained to an entirely new area through reasoning by analogy, putting forth hypotheses, reflecting on issues, and acting creatively.[10]

These skills are basic qualities for chemistry students and should be an essential and obvious constituent of the chemistry degree.[11] Still, these abilities often play a minor role in chemistry courses that often focus on subject-specific knowledge. For these reason, there has been great exertion to integrate "real-world" data-analysis activities into the chemical courses.

The pedagogical goal of this experiment is for students to develop an understanding of the redox process in chemical analysis with spectrophotometric data. The skills that are developed can then be exploited in more complex upper level experiments for chemical analysis.

MATERIALS AND METHODS

Equipment

A double beam UV−vis spectrophotometer (Cary 100 Bio, Varian, Inc.) with 10 mm quartz cells and Teflon lids was used for absorbance measurements. Another spectrophotometer that is able to measure absorbance at wavelength range 400−900 nm, equipped with 10 mm glass or plastic cuvettes can be used. Two milliliter multivolume transfer pipettes or 10−100-μL multivolume micropipettes were used.

Chemicals

Iron(III) nitrate nonahydrate CAS 7782-61-8 (Sigma-Aldrich, Poznań, Poland), potassium ferricyanide CAS 13746-66-2, sodium hydroxide CAS 1310-73-2 (POCH S.A., Gliwice, Poland), 6-propyl-2-thiouracil CAS 51-52-5 (99%, Sigma-Aldrich, Poznań, Poland), propylthiouracil 50 mg (Kali-ChemiePharma GMBH, Hannover, Germany), thyrosan 50 mg (SUN-FARM, Warsaw, Poland) and thyreostat II 25 mg (Dr. Herbrand KG, Gengenbach, Germany) were used.

All solutions were prepared with deionized water just before experiment.

Solutions of potassium ferricyanide and iron(III) nitrate (2×10^{-2} mol L^{-1}) were prepared by dissolving crystals in water.

To prepare 5×10^{-2} mol L^{-1} PTU standard solution, 0.0430 g of PTU was placed in a 5 mL volumetric flask containing 1 mL of 1 mol L^{-1} sodium hydroxide solution, and then the flask was diluted with deionized water to the mark, and effectively and carefully shaken. Next, 100 μL of the standard solution was transferred into a 5 mL volumetric flask containing 1 mL of deionized water, and then the flask was diluted with deionized water to the mark and carefully shaken to prepare 1×10^{-3} mol L^{-1} working PTU solution.

To prepare solutions of pharmaceuticals, 1/10 weight of one tablet (10 tablets were weighed, an average mass of one tablet was calculated, and one tablet was crushed in mortar) of thyrosan, propylthiouracil, or thyreostat II, measured accurately, was placed in a 100 mL volumetric flask containing 10 mL of 1 mol L^{-1} sodium hydroxide solution. The flask was placed in an ultrasonic bath and shaken for 10 min to accelerate the dissolution process. Finally, the sample was diluted to the mark with water.

EXPERIMENTAL PROCEDURE

Procedure for Establishing the Range of Determination and Calibration Curve

A total of 1.5 mL of 2×10^{-2} mol L^{-1} Fe^{3+} and 2 mL of 2×10^{-2} mol L^{-1} $[Fe(CN)_6]^{3-}$ solutions were placed in a 5 mL volumetric flask, followed by the addition of an appropriate volume of PTU working solution (Table 1).

Table 1. Scenarios Designed for Working Solution Preparation

Volume Taken [μL]	Weight Taken [μg]	Concentration of Final Solution [μg L^{-1}]
10	1.70	340
25	4.25	850
50	8.50	1700
75	12.75	2550
100	17.00	3400

The flask was left to stand for 30 min, and then the solution was diluted to the mark with water. Finally, the solution was transferred into a spectrophotometric cell and absorbance was measured at 840 nm (Table 1) against a blank solution containing all the reagents except the compound being determined.

Procedure for Determination of PTU in Pharmaceuticals

Estimation of PTU content in pharmaceuticals was conducted according to the general procedure described above. Clear 200 μL portions of drug solutions were used for analysis instead of PTU standard solution.

HAZARDS

Iron(III) nitrate and potassium ferricyanide cause eye and skin irritation. They are harmful if swallowed. 6-Propyl-2-thiouracil is not thought to produce harmful health effects. Prussian blue is not toxic, but stains from it can be hard to remove from surfaces, clothes, and skin if it spills. Students work with diluted solutions of reactants wearing lab coats, protective gloves, and eye protection. Waste materials are dispensed into containers following the guidelines of laboratory hazardous waste management.

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J. Chem. Educ. XXXX, XXX, XXX−XXX

RESULTS AND DISCUSSION

The first necessary step in the development of the method is the choice of optimal conditions.

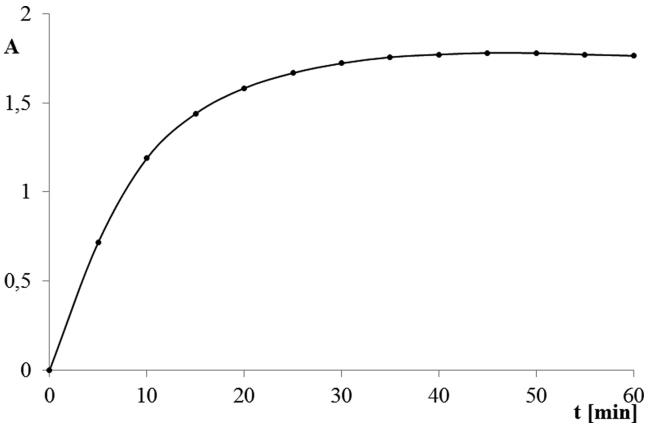

Figure 2. Relationship of absorbance with the changes of reaction time of 40 μmol $[Fe(CN)_6]^{3-}$, 30 μmol Fe^{3+}, 0.1 μmol PTU.

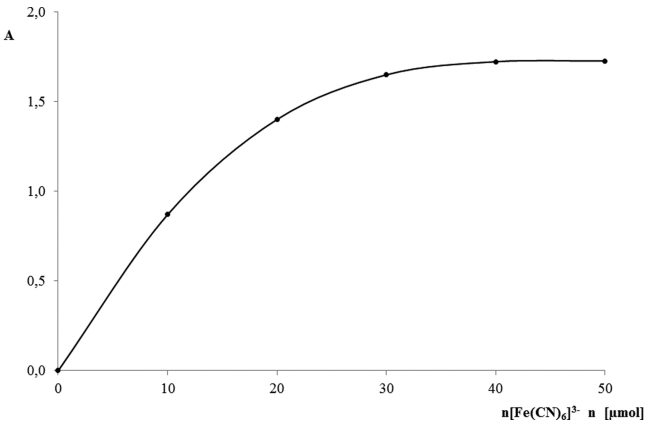

Figure 3. Relationship of absorbance with the changes in number of moles of $[Fe(CN)_6]^{3-}$ (5 μmol Fe^{3+}, 0.1 μmol PTU).

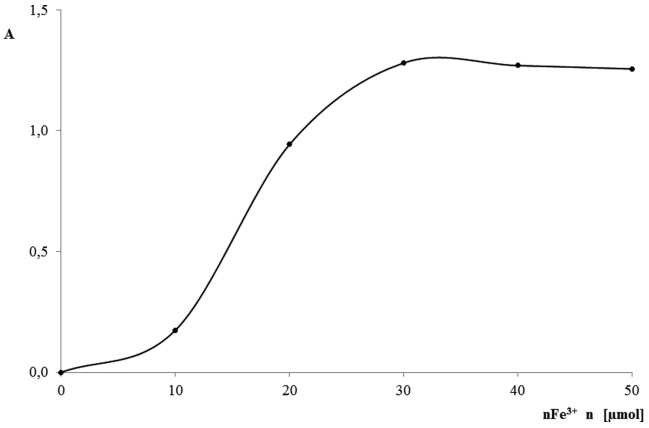

Figure 4. Relationship of absorbance with the changes in number of moles of Fe^{3+} (5 μmol $[Fe(CN)_6]^{3-}$, 0.1 μmol PTU).

The absorbance changes of the solutions depending on their composition in time were measured (Figure 2). The composition that ensures high absorbance value, and relatively short reaction time, was chosen as optimal. In the first step, the measurements of solutions that contained constant amount of Fe^{3+} (5 μmol) and PTU (0.1 μmol) while the amount of $[Fe(CN)_6]^{3-}$ was changing were performed (Figure 3). We found that the highest absorbance, and the shortest reaction time, was obtained for 40 μmol of $[Fe(CN)_6]^{3-}$. In the next step, absorbance changes of solutions consisting of 5 μmol of $[Fe(CN)_6]^{3-}$, 0.1 μmol of PTU, and different number of moles of Fe^{3+} were measured. The highest absorbance, and the shortest reaction time, was obtained for 30 μmol of Fe^{3+}. Thus, molar ratio of Fe^{3+} to $[Fe(CN)_6]^{3-}$ is 3:4. The molar ratio of Fe^{3+} to $[Fe(CN)_6]^{3-}$ depends on experimental conditions and the obtained value is according to literature.[12] As reaction time is constant from 30 min, the selected optimum time was 30 min (Figures 3 and 4).

On the basis of the absorption spectrum of the Prussian blue complex produced by the reaction of Fe^{3+} and $[Fe(CN)_6]^{3-}$ with 6-propyl-2-thiouracil (Figure 5), the analytical wavelength of maximum absorbance was also established (840 nm).

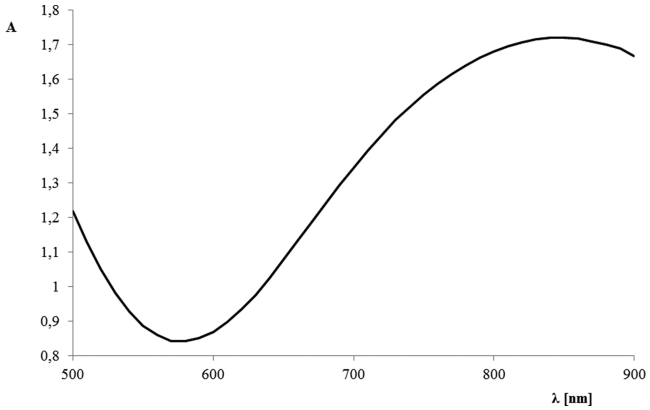

Figure 5. Absorption spectrum of the Prussian blue complex produced by the reaction of Fe^{3+} and $[Fe(CN)_6]^{3-}$ with 6-propyl-2-thiouracil: 30 μmol Fe^{3+}, 40 μmol $[Fe(CN)_6]^{3-}$, 0.1 μmol PTU (in V = 5 mL), t = 30 min.

Table 2. Results of 6-Propyl-2-thiouracil Determination

Weight Taken [μg]	Average absorbance	Found [μg]	RSD [%]
1.70	0.0995 ± 0.0016	1.61 ± 0.03	1.60
4.25	0.2682 ± 0.0008	4.38 ± 0.04	0.29
8.50	0.5217 ± 0.0011	8.54 ± 0.02	0.20
12.75	0.7783 ± 0.0013	12.76 ± 0.02	0.16
17.00	1.0361 ± 0.0007	16.99 ± 0.01	0.07

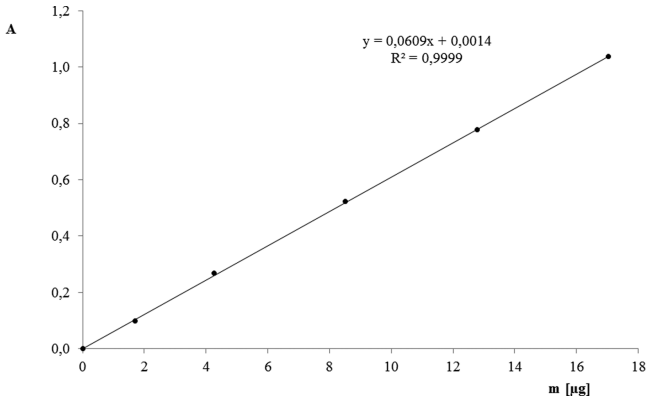

Figure 6. Calibration graph between absorbance and the amount of 6-propyl-2-thiouracil for its determination in pharmaceuticals.

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J. Chem. Educ. XXXX, XXX, XXX−XXX

Table 3. Results of 6-Propyl-2-thiouracil Determination in Pharmaceuticals

Drug	Declared content [mg]	Average weight of 1/10 mass of one tablet [mg]	Found [mg]	RSD [%]	Recovery [%]
Thyrosan	50	18.3	50.6 ± 0.10	0.20	101.1
Propylthiouracil	50	20.3	50.4 ± 0.3	0.58	100.7
Thyreostat II	25	22.2	24.8 ± 0.4	1.67	99.3

All measurements were conducted according to the procedure described for the calibration curve, changing the optimized parameters within the defined range.

Under optimal conditions, estimations of different amounts of the compound were performed, a calibration curve was constructed for the relationship between absorbance values and PTU amount in the sample, and the determination range was found (1.7−17 μg). Linearity was checked using the least-squares linear regression method. The regression equation was $y = 0.0609x + 0.0014$ (x, mass of PTU) with a determination coefficient $R^2 = 0.9999$. There is increase of RSD with the decreases of used concentration. The RSD generally decreases with the increasing concentration of an analyte. The expected trend indicates that analytical precision follows counting statistics and, thus, that most of the data variation is analytical in origin. The linear range of calibration curve is sufficiently large to encompass a determination of drug in pharmaceutical formulations. The results are presented in Table 2.

On the basis of these results, the limit of detection (LOD = $3s_b/a$ = 0.158 μg) and the limit of quantification (LOQ = $10s_b/a$ = 0.527 μg) were also calculated (s_b is standard deviation of the intercept and was calculated using Excel; a is the slope of the calibration curve).

The presented method was successfully applied for the estimation of the content of the compound in pharmaceutical formulations. Assays were performed using the calibration curve for the relationship between absorbance values and the amount of PTU in the sample (Figure 6).

With the use of the least-squares method, a linear regression equation was established and the content of PTU in drug tablets was calculated. Mass of PTU (m) calculated from the equation is contained in 200 μL portion of 100 mL solution which was prepared from 1/10 mass of one tablet. Thus, mass of PTU in one tablet is equal to 5m. The results presented in Table 3 show that the method is useful for this purpose, and is precise and accurate.

SUMMARY

This lab exercise aids to develop the student's understanding of complex skills such as mass proportions, molarity, dilutions, redox reactions of inorganic and organic compounds, and the relationship between absorbance and concentration as represented in the Beer−Lambert−Bouguer law. During the experiment, students collect experimental results and calculate the amount of a drug in pharmaceutical preparations. Teachers may transform the student handouts to make this need an inquiry-based laboratory investigation suitable for students finishing a laboratory course at a high school or university general chemistry level.[13]

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available on the ACS Publications website at DOI: 10.1021/ed400845q.

Handouts for the students (PDF, DOCX)

AUTHOR INFORMATION

Corresponding Author

*E-mail: robzak@chemia.uni.lodz.pl.

Notes

The authors declare no competing financial interest.

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J. Chem. Educ. XXXX, XXX, XXX−XXX